DESCRIPTION

SRSV DETECTION KIT

Technical Field

This invention relates to a kit for detecting and distinguishing one or more small round structure viruses (hereinafter called "SRSVs") in a specimen.

Background Art

SRSVs are a group of causative viruses of human viral gastroenteritis, the discovery of the first one of which goes back to 1972. They are known to cause infantile acute gastroenteritis and also outbreaks of food poisoning or the like among adults and preschool or elementary school children. Due to the inability to proliferate these SRSVs by cell culture and the lack of animal models capable of exhibiting sensitivity thereto, SRSV antigens and anti-SRSV antibodies are hardly available, resulting in a delay in the development of immunoserologic methods for the detection of the viruses.

Under such circumstances, it was succeeded to clone the gene of the Norwalk virus, an SRSV, in 1993, leading to the determination of the base sequence of its complete genomes [JP(PCT) 6-506823 A]. Subsequently, PCR methods which are useful to amplify a part of an RNA polymerase region were developed, and 14 SRSV-related viruses have been found to date. As a result

of analyses of about 120 amino acids in these RNA polymerase regions, SRSVs are considered to be roughly differentiated into two genogroups, that is, Genogroup I including the Norwalk virus strain as a prototype and Genogroup II including the Snow Mountain virus strain as a prototype.

As genetic analyses of SRSV-related viruses proceeded, it came to knowledge that substantial diversity exists even in the same genogroup. As a matter of fact, it was found that with an RT-PCR method making use of primers for the genes of the Norwalk virus and Snow Mountain virus strains as the prototypes of the respective genogroups, every SRSV is not detectable and also that it is very difficult to design primers or set RT-PCT conditions for achieving efficient amplification of SRSVs.

In the meantime, antigens were prepared against some of the viruses, such as the Norwalk virus strain and the Snow Mountain strain, by genetic expression, antibodies were obtained, and ELISA-dependent SRSV detection methods making use of such antibodies were also developed. It was, however, still impossible to detect every gastroenteritis-causing SRSV due to the diversity of the SRSVs.

In Japan, on the other hand, SRSVs were designated in 1997 to be causative factors of food poisoning as defined in the Food Sanitation Act so that, if SRSV food poisoning breaks out, determination of its infection route is required. There is accordingly a desire for a method which easily and surely detects

and identifies SRSVs in infected subjects' feces or foods.

Disclosure of the Invention

Accordingly, an object of the present invention is to provide a kit which can easily detect from a specimen an SRSV-related virus known to date and can surely discriminate its serotype and genogroup.

With the foregoing circumstances in view, the present inventors have proceeded with an genetic and immunological investigation on SRSV-related viruses. As a result, it has been found that combined use of antibodies obtained from 11 SRSV-related virus peptides, including newly-found novel virus peptides, can detect most SRSVs in specimens and can surely discriminate the serotypes and genogroups of the SRSVs, leading to the completion of the present invention.

Specifically, the present invention provides an SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

The present invention also provides an SRSV detection kit for discriminating SRSVs in genogroup, said SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (d), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Further, the present invention also provides an SRSV detection kit for discriminating genogroup of SRSVs, said SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide

groups (e) to (k), respectively:

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Furthermore, the present invention also provides SRSV-related virus strain genes having base sequences

represented by SEQ ID NOS: 15, 20, 21 and 22 or base sequences similar to the first-mentioned base sequences, respectively, except for deletion, replacement or addition of one to several bases of said first-mentioned base sequences.

Brief Description of the Drawings

FIG. 1 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Seto 124/1989/JP strain.

FIG. 2 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Funabashi 258/1996/JP strain.

FIG. 3 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chiba 407/1987/JP strain.

FIG. 4 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Narita 104/1997/JP strain.

FIG. 5 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Sanbu 809/1998/JP strain.

FIG. 6 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Ichikawa 754/1998/JP strain.

FIG. 7 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chitta 1876/1996/JP strain.

FIG. 8 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Kashiwa 47/1997/JP strain.

FIG. 9 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Mie 7k/1994/JP strain.

FIG. 10 is an electron micrograph (x 100,00) of virus-like

particles derived from the Hu/NLV/Kashiwa 645/1999/JP strain.

FIG. 11 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Osaka 10-25/1999/JP strain.

Best Modes for Carrying Out the Invention

1. SRSV-related viruses

The SRSV detection kit according to the present invention is characterized by the use of the antibodies against SRSV-related virus constituting peptides having the 11 specific amino acid sequences or at least 80% of homologies with the amino acid sequences in the groups (a) to (k). Of these, the peptides belonging to the group (d), the group (i), the group (j) and the group (k) are novel peptides different from any SRSV-related viruses registered with the GeneBank to date (Table 1, which will be described subsequently herein). Owing to the incorporation of the 11 antibodies, including antibodies against these novel peptides, into the kit, SRSV-related viruses can be detected without omission.

The SRSV-related virus constituting peptides useful in the present invention embrace their mutants in each of which one or more amino acids have been deleted from, replaced in or added to its corresponding amino acid sequence; and also their mutants in each of which one or several bases have been deleted from, replaced in or added to a base sequence encoding its corresponding amino acid sequence.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 1 in the group (a) is a virus constituting peptide of the Hu/NLV/Kashiwa 645/1999/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Desert Shield/90/SA strain (GeneBank Accession No. U04469).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 2 in the group (b) is a virus constituting peptide of the Hu/NLV/Seto 124/1989/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the KY-89/89J strain (GeneBank Accession No. L23828) and the Norwalk/68/US strain (GeneBank Accession No. M876611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 3 in the group (c) is a virus constituting peptide of the Hu/NLV/Funabashi 258/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Southampton/91/UK strain (GeneBank Accession No. L07418).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 4 in the group (d) is a virus constituting peptide of the Hu/NLV/Chiba 407/1987/JP strain

obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 4 has less than 75% of homology in structural gene (SEQ ID NO: 15) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 5 in the group (e) is a virus constituting peptide of the Hu/NLV/Narita 104/1997/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Bristol/93/UK strain (GeneBank Accession No. X76716), the Lordsdale/93/UK strain (GeneBank Accession No. X86557), and the Camberwell/94/AU strain (GeneBank Accession No. U46500).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 6 in the group (f) is a virus constituting peptide of the Hu/NLV/Sanbu 809/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Mexico/89/MEX strain (GeneBank Accession No. U22498), the Auckland strain (GeneBank Accession No. U460391), the Toronto/77/CA strain (GeneBank Accession No. U02030), and the

OTH-25/89/J strain (GeneBank Accession No. L23830).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 7 in the group (g) is a virus constituting peptide of the Hu/NLV/Ichikawa 754/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Snow Mountain/76/US strain (GeneBank Accession No. U70059) and the Melksham/89/UK strain (GeneBank Accession No. X81879).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 8 in the group (h) is a virus constituting peptide of the Hu/NLV/Chitta 1876/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Hawaii/71/US strain (GeneBank Accession No. U07611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 9 in the group (i) is a virus constituting peptide of the Hu/NLV/Kashiwa 47/1997/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 9 has less than 75% of homology in structural gene (SEQ ID NO: 20) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence

not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 10 in the group (j) is a virus constituting peptide of the Hu/NLV/Mie 7k/1994/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 10 has less than 70% of homology in structural gene (SEQ ID NO: 21) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 11 in the group (k) is a virus constituting peptide of the Hu/NLV/Osaka 10-25/1999/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 11 has less than 70% of homology in structural gene (SEQ ID NO: 22) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Table 1

Virus strain	GeneBank Accession No.
Desert Shield/90/SA	U04469
Norwalk/68/US	M876611
KY-89/89J	L23828
OTH-25/89/J	L23830
Southampton/91/UK	L07418
Lordsdale/93/UK	X86557
Bristol/93/UK	X76716
Camberwell/94/AU	U46500
Toronto/77/CA	U02030
Mexico/89/MEX	U22498
Snow Mountain/76/US	U70059
Melksham/89/UK	X81879
Auckland	U460391
Hawaii/71/US	U07611

The SRSV-related virus constituting peptides in these groups (a) to (k) embrace, in addition to the above-described peptides, partial peptides each of which contains a specific amino acid sequence in its corresponding peptide and has antigenecity equivalent to the corresponding peptide.

According to a homological analysis of about 120 amino acids of RNA polymerase regions of the SRSV-related virus constituting peptides, these SRSV-related virus constituting peptides can be classified into two genogroups. Described

specifically, they can be classified into Type I to which the peptides in the groups (a) to (d) belong and Type II to which the peptides in the groups (e) to (k) belong.

2. Cloning of the SRSV-related virus constituting genes

From feces of an SRSV infected patient, viral RNA is extracted using the cetyltrimethylammonium bromide (CTAB) method or the like, cDNA was formed by an oligo-dT primer and a reverse transcriptase, and using the cDNA and primers capable of amplifying structural gene regions of the individual SRSV-associate viruses, PCR was conducted to amplify structural gene fragments.

Such a structural gene fragment is inserted in a plasmid by once conducting TA cloning with an *E. coli* cloning vector.

As a cloning vector usable here, it is possible to use a known cloning vector such as a vector derived from a plasmid obtained using as host procaryotic cells represented by *E. coli* or from a bacteriophage represented by ë phage, and appropriately combined use of a cloning vector and its host cell is desired. Specific examples of the cloning vector include pBR322, pUC19 and pCRII. The insertion of the DNA can be conducted by a method known *per se* in the art, and upon formation of such a vector, use of *E. coli* cells is desired as they permit easy genetic manipulation.

3. Expression of structural gene and creation of virus-like particles.

By having fragments of the above-obtained individual virus constituting genes in the groups (a) to (k) expressed with a suitable expression system or by using virus-like particles created from the virus constituting peptides in a genetic engineering manner, antibodies against the respective viruses can be obtained. A description will hereinafter be made about an expression when *E. coli* is used and also about the creation of virus-like particles.

(1) Expression by *E. coli*

Plasmids with the structural gene regions of the respective SRSV-related viruses incorporated therein, respectively, are each digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in pGEX (GST fusion protein expression vector; product of Pharmacia AB), pTrc99A (*E. coli* expression vector; product of Pharmacia AB), pTrxFus (thioredoxin fusion protein expression vector; product of Invitrogen Corporation), pET (expression vector making use of pT7RNA promoter; product of Novagen Inc.), a maltose binding protein expression vector, or a â galactosidase fusion protein expression vector. At this time, the structural gene region to be incorporated can be of the complete length or can be a partial region, with a partial region containing at least one antigen epitope of an SRSV being preferred. Gene expression vectors with the structural gene regions incorporated therein

as described above are transformed by an *E coli* strain suited for gene expression, for example, the BL21 strain, the DH10B strain, the JM109 strain or the XL1-Blue strain. Expression of the gene can be conducted by culturing the thus-obtained transformants in a general liquid culture medium, for example, L-broth. It is preferred for the expression to add a gene expression promoter, for example, IPTG or, when a PL promoter is used, to apply a heat shock.

Purification of a peptide so expressed can be conducted following a general purification method for expressed protein, which makes use of *E coli*. If the expressed protein is in a dissolved form, for example, its purification can be conducted by affinity chromatography making use of a GST column or a column for maltose binding proteins. If the expressed protein is in an insoluble form, its purification can be achieved by conducting affinity chromatography making use of a Ni chelate.

(2) Creation of SRSV virus-like particles

A plasmid with a structural gene region of an SRSV-related virus incorporated therein is digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in a baculovirus transfer vector such as pVL1393. The transfer vector and a linear baculovirus DNA, from which a gene region essential for proliferation has been deleted, are subjected to transfection in insect cells such that homologous

recombination is induced to form the target recombinant baculovirus.

By infecting the thus-obtained recombinant baculovirus to insect cells such as Sf9 cells or Tn5 cells and incubating the infected insect cells under adequate growth conditions in a manner known *per se* in the art, the structural protein of the SRSV is expressed. By allowing the structural protein to undergo self-assembly, virus-like particles can be produced. Use of a biochemical purification method, for example, centrifugation makes it possible to isolate and purify the virus-like particles. Whether or not such virus-like particles have been formed can be confirmed by subjecting the self-assembled product to negative staining with uranyl acetate and examining the stained self-assembled product by an electron microscope.

The virus-like particles obtained as described above do not have infectiveness as they do not contain any gene internally. Nonetheless, they have antigenecity equivalent to virus particles because they structurally have substantially the same shape as virus particles.

4. Acquisition of antibodies against SRSV-related viruses

By immunizing an animal with the thus-obtained virus constituting peptide or virus-like particles, an anti-SRSV-related virus antibody can be prepared. Incidentally, such an antibody can be either a monoclonal antibody or a polyclonal antibody.

Preparation of an immune antibody by making use of virus-like particles can be conducted, for example, as will be described next. In a manner known *per se* in the art, a rabbit is immunized with virus-like particles of one of the SRSV-related viruses, and from separated serum, an IgG antibody (anti-SRSV antibody) against the virus-like particles can be obtained. For the separation and isolation of the antibody, a method such as DEAE Sepharose chromatography can be used.

Using the 11 types of virus-like particles of the groups (a) to (k) obtained as described above and their corresponding anti-SRSV antibodies, their cross reactivities were measured. As will be shown below in Table 2, absolutely no cross-reactivity was exhibited between the individual SRSV-related viruses. According to the SRSV detection method of the present invention, it is therefore possible to concurrently discriminate the serotypes of 11 types of SRSVs. This also indicates the possibility of discriminating Genogroup I Genogroup II from each other at the same time.

5. Detection of SRSV-related viruses

For the detection of one or more SRSVs in a specimen by the individual anti-SRSV antibodies obtained as described, conventionally employed immunoassays making use of antigen-antibody reactions, for example, radioimmunoassay by the sandwich technique, enzyme-linked immunosorbent assay (ELISA) and the like can be used, with ELISA being particularly

preferred. Described specifically, the 11 types of anti-SRSV antibodies are separately poured into a microplate to prepare an SRSV screening plate. A dilution of a fecal emulsion, which has been prepared from feces of an SRSV infected patient, is added to the wells of the plate, and is then allowed to react. Peroxidase (POD) labeled anti-SRSV antibodies of the respective viruses are thereafter added and reacted. After a substrate solution (TMB containing hydrogen peroxide) is added and reacted, 0.6 N sulfuric acid is added to stop the reactions. By measuring the absorbance (450 nm/630 nm) of each well by an ELISA autoreader, the SRSV or SRSVs can be detected.

When it is desired to conduct only the detection of one or more SRSVs in a specimen, a detection kit can be prepared by using a microplate with all the 11 types of anti-SRSV antibodies mixed and immobilized thereon. To also discriminate even the serotypes of the one or more SRSVs, a detection kit can be prepared by using microplates with all the 11 types of anti-SRSV antibodies immobilized separately thereon.

Further, the discrimination of the genogroups is feasible by a kit making use of a microplate with antibodies against the peptides in the groups (a) to (d) mixed and immobilized thereon (Type I plate) or a microplate with antibodies against the peptides in the groups (e) to (k) mixed and immobilized thereon (Type II plate).

Moreover, immobilization of the individual anti-SRSV

antibodies useful in the present invention with a carrier such as a latex or magnetic beads makes it possible to surely capture one or more SRSV-related viruses in a specimen. The carrier with one or more SRSV-associate viruses captured thereon can be recovered by centrifugation in the case of the latex or by magnet in the case of the magnetic beads. Subsequent to the recovery, virus RNAs can be extracted and used.

Examples

The SRSV detection kits according to the present invention will hereinafter be specifically described based on Examples.

Example 1 Cloning of structural genes of SRSV-related viruses

(1) Synthesis of cDNA

PBS (9 mL) and "Daiflon" (1 mL) were added to feces (0.5 to 1.0 g) of an SRSV patient, followed by homogenization. The homogenate was then centrifuged at 3,000 rpm for 20 minutes, and the supernatant was collected as a 10% fecal emulsion.

Using a 1-mL aliquot of the fecal emulsion, RNA of the SRSV was extracted by the cetyltrimethylammonium bromide (CTAB) method, and the RNA was eventually suspended in a 0.1% diethyl pyrocarbonate solution (30 iL). Using the suspension, cDNA was prepared by a reverse transcriptase derived from the Oligo-dT(12-18) primer and AMV (Avian Myeloblastosis Virus) (product of SEIKAGAKU CORPORATION).

(2) Isolation of structural gene regions

Using the cDNA prepared in (1) and primers for amplifying the structural gene regions shown below, PCR was conducted. Subsequent to the PCR, amplified structural gene fragments were separated by agarose gel electrophoresis, and were then recovered by using "SuprecTM-01" (TAKARA).

Hu/NLV/Kashiwa 645/1999/JP gene:G1/F2(SEQ ID NO: 23), Oligo-dT(33) (SEQ ID NO: 24)

Hu/NLV/Seto 124/1989/JP gene:G1/F2(SEQ ID NO: 23),G1/R0 (SEQ ID NO: 25)

Hu/NLV/Funabashi 258/1996/JP gene:G1/F2(SEQ ID NO: 23), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Chiba 407/1987/JP gene:D5(SEQ ID NO: 26), CV-U4(SEQ ID NO: 27)

Hu/NLV/Narita 104/1997/JP gene:97k104/F1(SEQ ID NO: 28), 97k104/R1(SEQ ID NO: 29)

Hu/NLV/Sanbu 809/1998/JP gene:G2/F3(SEQ ID NO: 30), MV-R1(SEQ ID NO: 31)

Hu/NLV/Ichikawa 754/1998/JP gene:G2/F3(SEQ ID NO: 30), SMV-R1(SEQ ID NO: 32)

Hu/NLV/Chitta 1876/1996/JP gene:G2/F3(SEQ ID NO: 30), G2/R0(SEQ ID NO: 33)

Hu/NLV/Kashiwa 47/1997/JP gene:97k104/F1(SEQ ID NO: 28), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Mie 7k/1994/JP gene:G2/F3(SEQ ID NO: 30), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Osaka 10-25/1999/JP gene:GFCR7(SEQ ID NO: 34), Oligo-dT(33)(SEQ ID NO: 24)

(3) Cloning of structural genes

TA cloning of the recovered structural gene fragments to an *E. coli* cloning vector, pCRII(product of Invitrogen Corporation) was conducted. Obtained from these clones were plasmids with the structural genes of the viruses incorporated therein, pCRII/645, pCRII/124, pCRII/258, pCRII/Chiba, pCRII/104, pCRII/809, pCRII/754, pCRII/76, pCRII/47, pCRII/7k, and pCRII/10-25.

Example 2 Determination of base sequences

Determination of the base sequences of the structural genes of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain was conducted in the below-described manner.

Firstly, a primer (first primer) was set in the vicinity of the polyhedrin promoter of pVL1393 as a transfer vector, and by the dye termination method, a labeling reaction was conducted by using a "Cycle Sequencing Kit FS" (product of Perkin-Elmer

Corp.). The DNA concentration of the transfer vector employed was 0.4 ig/iL, whereas the concentration of the sequencing primer used was 3.2 pmol/iL. Subsequent to the reaction, the excess fluorescent pigment was eliminated using a centriprep spin column (manufactured by Perkin-Elmer Corp.). The reaction mixture was completely dried by a vacuum lyophilizer, and the lyophilizate was suspended in a special sample buffer (20 iL; product of Perkin-Elmer Corp.). Subsequent to stirring, the suspension was subjected to centrifugal precipitation. The precipitate was dried at 95℃ for 2 minutes. After quenching, it was analyzed by an autosequencer ("ABI Genetic Analyzer 310").

Using the base sequence determined by the first primer, a new sequencing primer (second primer) was set on the 3'-side of the base sequence. Using this second primer, a labeling reaction was conducted by a cyclic sequencing kit in a similar manner as mentioned above. Subsequent to the reaction, operation similar to that mentioned above was performed, and the base sequence was analyzed by the autosequencer. As has been described above, a sequencing primer was set on the 3' side of the base sequence determined in each cycle, and determination of the base sequence was conducted. By repeating this procedure, the base sequences from the 5'-ends to the 3'-ends of the 11 types of SRSV-related virus structural genes (SEQ ID NO: 12 to SEQ ID NO 22) were determined. Among these, the base sequences represented by SEQ ID NO: 15 (the Hu/NLV/Chiba 407/1987/JP

strain), SEQ ID NO: 20 (the Hu/NLV/Kashiwa 47/1997/JP strain), SEQ ID NO: 21 (the Hu/NLV/Mie 7k/1994/JP strain) and SEQ ID NO: 22 (the Hu/NLV/Osaka 10-25/1999/JP strain) were confirmed to be novel sequences not reported to date.

Example 3 Creation of recombinant baculovirus capable of yielding virus-like particles

(1) Construction of transfer vectors

The plasmids with the structural gene regions incorporated therein, which had been obtained in Example 1(3), were digested by a restriction endonuclease which does not cleave the structural gene regions. Subsequent to separation by agarose gel electrophoresis, the structural gene regions were recovered by "SuprecTM01" (TAKARA). The recovered gene fragments were incorporated in baculovirus transfer vectors pVL1393 (product of Invitrogen Corporation), which had been digested by the same restriction endonuclease, to prepare transfer vectors.

(2) Creation of recombinant baculoviruses

Baculovirus DNA (0.5 ig; "Baculo-Gold") and one of the transfer vectors (1 ig) obtained in (1) were dissolved in distilled water (8 iL). The resulting solution was mixed with a two-fold dilution of lipofectin (equivalent amount), and the thus-obtained mixture was left over at room temperature for 15 minutes. After Sf9 cells (1×10^5 cells) suspended in an insect cell culture medium, "Ex-cell 400", were adsorbed at 26.5℃ for 30 minutes in a plastic Petri dish (diameter: 3.5 cm), a mixture

of the transfer vector and "Baculo-Gold" was added dropwise to the cells, followed by incubation at 26.5℃. 24 Hours later, the culture medium was replaced by a "TC100" (product of GIBCO BRL Life Technologies; hereinafter referred to as "TC100") which contained 10% fetal bovine serum and 2% BTB (products of GIBCO BRL Life Technologies), and incubation was continued further.

(3) Purification of recombinant baculoviruses

After each recombinant baculovirus obtained in (2) was incubated for 5 days, the culture supernatant was diluted tenfold with an insect cell culture medium such as TC100. A 0.1-mL aliquot of the diluted supernatant was taken, and inoculated to 3 x 10^6 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME (low melting-point agarose) was overlayed, followed by incubation at 26.5℃. On the 4^{th} day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was further overlayed, followed by incubation at 26.5℃. On the following day, the formed plaques were scraped off with a microtip. and were suspended in TC100 culture medium.

(4) Production of recombinant baculovirus seeds and measurement of their infective potencies

Each suspension obtained in (3) was inoculated to 1 x 10^7 Sf9 cells. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 was added, followed by incubation at 26.5℃ for 3 to 4

days. The culture was centrifuged at 2,500 rpm for 10 minutes at 4℃, and the culture supernatant was collected. The collected culture supernatant was inoculated to 1 x 10^7 Sf9 cells. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 was added, followed by incubation at 26.5℃ for 3 to 4 days.

Next, the culture supernatant was inoculated to 3 x 10^7 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME (low melting-point agarose) was overlayed, followed by incubation at 26.5℃. On the 4th day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was then overlayed, followed by incubation at 26.5℃. On the following day, the formed plaques were measured to calculate the infective potency of the recombinant baculovirus. This was recorded as the infective potency of the recombinant baculovirus.

Example 4 Creation of virus-like particles

(1) Expression of structural proteins by using recombinant baculoviruses

Each recombinant baculovirus was infected at M.O.I.s (Multiplicities of infection) of 1 to 10 to Sf9 insect cells. Upon infection, a suspension of the recombinant baculovirus was added dropwise to the cells, and the recombinant baculovirus was subjected to adsorption for about 60 minutes or so with gentle shaking. After that, TC100 was added as an insect cell culture

medium, followed by incubation at 26.5℃ for 5 to 6 days.

(2) Identification of expressed proteins

The culture supernatant of each recombinant virus infection was periodically sampled. After having been resolved by SDS-PAGE, the protein was detected by Coomassie blue staining, and by an expected molecular weight, the validity of the expressed protein was confirmation. Further, subsequent to resolving the protein by SDS-PAGE, the protein was transferred onto a nitrocellulose membrane, and by the Western blotting technique, the expressed protein was then identified with a convalescent serum of the SRSV.

(3) Purification and recovery of virus-like particles

The recombinant baculovirus seeds were infected at M.O.I.s of from 1 to 10. Subsequent to adsorption for about 60 minutes, "Ex-cell 400" was added, followed by incubation at 26.5℃ for 3 days. A protease inhibitor, for example, pepstatin A or a leupeptin, was then added to the culture to a final concentration of 1 mM, followed by further incubation for 2 to 3 days.

Subsequent to the incubation, the culture was centrifuged at 2,500 rpm for 10 minutes at 4℃ to collect the culture supernatant. The collected culture was centrifuged at 10,000 rpm for 30 minutes to eliminate the recombinant baculovirus. The supernatant was centrifuged at 25,000 rpm for 4 hours on a "Beckmann SW28 Rotor" to have virus-like particles precipitated. Then, the centrifuge tube from which the

supernatant had been discarded was held upside down to complete eliminate the supernatant. After that, Grace buffer or PBS(-) (0.5 mL) with the protease inhibitor added therein was added to the centrifuge tube, and the centrifuge was allowed to stand overnight at 4℃.

After the standing, the virus-like particles were suspended in the protease-inhibitor-containing Grace buffer which had been added, and were recovered. To the recovered virus-like particles, protease-inhibitor-containing Grace buffer or PBS(-) with CsCl (3.8 g) added therein was added to give 13 mL. The resulting mixture was ultracentrifuged at 16℃ and 35,000 rpm for 24 to 48 hours. Subsequent to the ultracentrifugation, a pale band in which virus-like particles gathered was collected. After 5-fold dilution with protease-inhibitor-containing Grace buffer, the resultant suspension was ultracentrifuged at 45,000 rpm for 3 hours on a "Beckmann TL100.3 Rotor" to have the virus-like particles precipitated.

The precipitated virus-like particles were solubilized with Grace buffer or PBS(-) to which the protease inhibitor had been added. Protease-inhibitor-containing Grace buffer solutions which contained 10% to 50% of sucrose were prepared in a 4PA tube, into which the solubilized solution of the virus-like particles was overlayed, followed by sucrose density-gradient centrifugation at 35,000 rpm for 4 hours at

4℃. Subsequent to the centrifugation, a pale band of virus-like particles was collected as purified SRSV virus-like particles in a 1-mL syringe fitted with a 26G needle.

The purified SRSV virus-like particles was diluted with Grace buffer as needed, and the quantity of protein was measured by the Bradford method.

The purified SRSV virus-like particles were subjected to negative staining with uranyl acetate, and were then examined by an electron microscope to ascertain whether or not virus-like particles had been formed (FIGS. 2 to 12).

Example 5 Preparation of Immune Antibodies and Labeled by Use of Antibodies Virus-like Particles

(1) Preparation of immune antibodies against virus-like particles

A phosphate buffer (pH 7.2, 1 mL) - which contained the purified SRSV virus-like particles (500 ig) obtained from one of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain - and the Freund's incomplete adjuvant (1 mL) were mixed, and then immunized to a New Zealand white rabbit

(3 kg) in a manner known *per se* in the art. Three weeks later, the rabbit was immunized further with a mixture of a phosphate buffer (pH 7.2, 1 mL), which contained the SRSV virus-like particles (0.25 ig), and the Freund's incomplete adjuvant (1 mL) (booster dose). Additional 3 weeks later, immunization was conducted as in the booster dose, and about 7 to 10 days after the additional booster dose, exsanguination was conducted, and the serum component was separated.

After the separated and purified serum was subjected to ammonium sulfate fractionation, the relevant fraction was dialyzed overnight at 4℃ against 50 mM Tris-HCl (pH 7.6). The inner dialyzate was then subjected to DEAE Sepharose chromatography which had been equilibrated with 50 mM Tris-HCl (pH 7.6). Under monitoring at an UV wavelength of 280 nm, an O.D. peak was collected to obtain an DEAE-purified IgG antibody (anti-SRSV antibody) against the virus-like particles.

(2) Preparation of labeled antibodies

Each anti-SRSV antibody was labeled with POD by an improved periodic acid technique ["Koso Men-eki Sokuteiho (Enzyme Immunoassay)", 2, 91, 1982]. Described specifically, POD was dissolved at 4 mg/mL in distilled water and 0.1 M sodium periodate (0.2 mL) was added, followed by a reaction at room temperature for about 20 minutes. The reaction mixture was then dialyzed overnight against 1 mM sodium acetate buffer (pH 4.0). Subsequent to the dialysis, 0.2 M sodium carbonate buffer (pH

9.5, 0.02 mL) was added to adjust the pH to 9.5, and at the same time, the anti-SRSV antibody (8 mg) was added.

After having been allowed to react at room temperature for 2 hours, 4 mg/mL sodium borohydroxide (0.1 mL) was added, followed by a reaction at 4℃ for about 2 hours. After the reaction, gel filtration was conducted with "Sephacryl S-200" while using 10 mM phosphate buffer. Under monitoring at an UV wavelength of 280 nm, a POD-labeled anti-SRSV antibody fraction was collected.

(3) Preparation of a solid-phase anti-SRSV antibody microplate

The anti-SRSV antibodies were separately diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL and then poured at 100 iL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was then allowed to stand overnight 4℃. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 iL/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight 4℃ to obtain a solid-phase anti-SRSV antibody microplate.

Example 6 Cross-reactivity

(1) Antigen detection ELISA

The purified SRSV virus-like particles of each group were diluted to 4 ng/mL to 0.04 ng/mL with a solution containing bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.2% and 0.05%, respectively, in a buffer (10 mM PBS, pH 7.2).

Then, the diluted emulsions of the virus-like particles (VLPs) were each added at 100 iL/well to wells of the corresponding solid-phase anti-SRSV antibody microplate, followed by a reaction at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction. 10 mM PBS (pH 7.2) which contained "Tween 20" at a final concentration of 0.05% was added at 200 iL/well to the wells, and was then eliminated under suction likewise. This procedure was repeated at least three times. After washing, the POD-labeled anti-SRSV antibody of the corresponding serotype, which had been diluted 20000-fold with a buffer, was added at 100 iL/well, followed by a reaction at room temperature for 60 minutes. Subsequent to washing, a TMB solution with hydrogen peroxide contained therein was added at 100 iL/well, followed by a reaction at room temperature for 30 minutes. After the reaction, 0.6 N sulfuric acid was added at 100 iL/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader. The results are shown in Table 2.

Table 2 Cross-reactivity between Serotypes

Purified VLP	VLP concent-ration (ng/mL)	Solid-phase antibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)										
		124 20000	258 20000	407 20000	645 20000	104 20000	809 20000	754 20000	1876 20000	47 20000	7k 20000	10-25 20000
124	4	1.430	0.018	0.013	0.016	0.013	0.007	0.007	0.008	0.010	0.019	0.009
	0.4	0.192	0.011	0.010	0.011	0.014	0.007	0.007	0.008	0.011	0.018	0.009
	0.04	0.030	0.011	0.011	0.011	0.013	0.007	0.007	0.009	0.012	0.018	0.009
258	4	0.042	1.831	0.114	0.020	0.015	0.009	0.007	0.010	0.012	0.019	0.010
	0.4	0.013	0.270	0.022	0.013	0.016	0.009	0.007	0.009	0.013	0.019	0.011
	0.04	0.008	0.043	0.012	0.011	0.017	0.008	0.007	0.009	0.012	0.018	0.010
407	4	0.084	0.045	0.974	0.010	0.015	0.007	0.007	0.009	0.011	0.018	0.009
	0.4	0.016	0.012	0.134	0.010	0.013	0.007	0.008	0.009	0.011	0.018	0.009
	0.04	0.009	0.010	0.025	0.011	0.014	0.007	0.007	0.008	0.011	0.019	0.009
645	4	0.149	0.034	0.023	0.320	0.016	0.008	0.008	0.009	0.011	0.020	0.010
	0.4	0.024	0.013	0.012	0.045	0.017	0.009	0.008	0.009	0.012	0.019	0.011
	0.04	0.010	0.010	0.011	0.014	0.015	0.009	0.008	0.008	0.012	0.021	0.011
104	4	0.007	0.009	0.009	0.010	0.708	0.007	0.015	0.025	0.017	0.031	0.009
	0.4	0.010	0.009	0.009	0.010	0.094	0.008	0.008	0.011	0.013	0.020	0.009
	0.04	0.009	0.009	0.010	0.011	0.024	0.008	0.007	0.009	0.012	0.020	0.009
809	4	0.013	0.012	0.012	0.011	0.114	0.877	0.047	0.143	0.046	0.080	0.017
	0.4	0.010	0.010	0.011	0.011	0.030	0.134	0.013	0.033	0.018	0.028	0.013
	0.04	0.009	0.010	0.010	0.010	0.017	0.022	0.008	0.011	0.014	0.020	0.011
754	4	0.008	0.011	0.009	0.010	0.038	0.008	0.286	0.068	0.025	0.027	0.013
	0.4	0.008	0.009	0.010	0.011	0.017	0.008	0.038	0.015	0.013	0.020	0.010
	0.04	0.009	0.009	0.011	0.011	0.016	0.008	0.011	0.010	0.012	0.020	0.009
1876	4	0.010	0.012	0.011	0.011	0.026	0.009	0.013	0.728	0.023	0.025	0.012
	0.4	0.009	0.014	0.010	0.011	0.017	0.009	0.008	0.089	0.015	0.021	0.013
	0.04	0.011	0.010	0.010	0.012	0.016	0.010	0.007	0.017	0.014	0.019	0.011
47	4	0.008	0.009	0.009	0.010	0.017	0.007	0.008	0.011	0.324	0.021	0.014
	0.4	0.008	0.009	0.009	0.011	0.015	0.008	0.008	0.009	0.048	0.020	0.013
	0.04	0.008	0.009	0.009	0.011	0.014	0.008	0.008	0.008	0.017	0.022	0.011

Table 2 (Cont'd)

Purified VLP	VLP concent-r ation (ng/mL)	Solid-phase amtibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)										
		124 20000	258 20000	407 20000	645 20000	104 20000	809 20000	754 20000	1876 20000	47 20000	7k 20000	10-25 20000
7k	4	0.009	0.010	0.010	0.011	0.019	0.009	0.010	0.011	0.015	0.160	0.014
	0.4	0.009	0.011	0.010	0.011	0.016	0.008	0.008	0.008	0.015	0.035	0.016
	0.04	0.011	0.010	0.010	0.011	0.017	0.009	0.008	0.009	0.014	0.022	0.015
10-25	4	0.009	0.010	0.010	0.011	0.098	0.010	0.022	0.069	0.033	0.058	1.050
	0.4	0.007	0.009	0.010	0.011	0.026	0.009	0.009	0.020	0.018	0.026	0.163
	0.04	0.009	0.009	0.009	0.012	0.016	0.009	0.007	0.011	0.015	0.023	0.029
Blank		0.009	0.011	0.010	0.011	0.016	0.009	0.008	0.009	0.017	0.022	0.016

In the table, "645" indicates the Hu/NLV/Kashiwa 645/1999/JP strain, "124" the Hu/NLV/Seto 124/1989/JP strain, "258" the Hu/NLV/Funabashi 258/1996/JP strain, "407" the Hu/NLV/Chiba 407/1987/JP strain, "104" the Hu/NLV/Narita 104/1997/JP strain, "809" the Hu/NLV/Sanbu 809/1998/JP strain, "754" the Hu/NLV/Ichikawa 754/1998/JP strain, "1876" the Hu/NLV/Chitta 1876/1996/JP strain, "47" the Hu/NLV/Kashiwa 47/1997/JP strain, "7k" the Hu/NLV/Mie 7k/1994/JP strain, and "10-25" the Hu/NLV/Osaka 10-25/1999/JP strain.

As a result, no cross-reactivity was observed between viruses of the same genogroup, to say nothing of cross-reactivity between viruses of different Genogroups I and II. It was, therefore, confirmed that the serotypes of the 11 types of used virus strains were different from one another.

Test 1 Discrimination of SRSVs in Genogroup

The anti-SRSV antibodies against the SRSVs belonging to Genogroup I (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, and the Hu/NLV/Chiba 407/1987/JP strain) were diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL and were then mixed. The thus-obtained mixture was poured at 100 iL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4℃. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 iL/well with PBS which contained "Tween 20" at a final concentration of 0.05%.

10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight at 4℃ to obtain a microplate with the anti-SRSV-IgG antibodies against the respective serotypes of Genogroup I carried in a mixed solid-phase form (Type I plate).

Next, the anti-SRSV antibodies against the SRSVs belonging to Genogroup II (the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were similarly formed into a solid phase to obtain a Type II plate.

To feces (0.5 to 1.0 g) of each SRSV patient, PBS (9 mL) and "Daiflon" (1 mL) were added, followed by homogenization. The thus-prepared suspension was centrifuged under 19,000 g for 20 minutes, and the supernatant was collected and formed into a 10% fecal emulsion. The 10% fecal emulsion was diluted at 1:1 in volume with a buffer. The diluted emulsion was added at 100 iL/well into wells of the Type I and Type II plates, and was allowed to react at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction. 10 mM PBS (pH 7.2) - which contained "Tween 20" at a final concentration of 0.05% - was added at 200 iL/well to the wells, and was then eliminated under suction. This

procedure was performed at least three times. After the washing, the POD-labeled anti-SRSV antibodies of the respective serotypes, said antibodies having had been diluted 20,000-fold with a buffer, were added at 100 iL/well, and were then reacted at room temperature for 60 minutes. After washing, a TMB solution with hydrogen peroxide contained therein was added at 100 iL/well, and were then reacted at room temperature for 30 minutes. Subsequent to the reaction, 0.6 N sulfuric acid was added at 100 iL/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader.

As a result, it was found that among 15 fecal specimens from patients infected to SRSV of Genogroup I, 14 fecal specimens reacted only to the Type I plate and did not react to the Type II plate. Concerning 7 fecal specimens from patients infected to SRSV of Genogroup II, on the other hand, 6 fecal specimens did not react to the Type I plate but reacted only to Type II plate. It has, therefore, been confirmed that discrimination in genogroup is actually feasible.

Test 2 Discrimination of SRSVs in Serotype

The anti-SRSV antibodies against the SRSVs (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the

Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL. The thus-obtained dilutions were poured at 100 iL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4℃. After having been allowed to stand for 18 hours or longer, the microplate was washed 3 to 4 times at 200 iL/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight at 4℃ to obtain a solid-phase anti-SRSV antibody microplate (serotype discrimination plate).

With respect to fecal specimens from SRSV patients, ELISA was conducted in a similar manner as in Test 1. The results are shown in Table 3.

Table 3 Clinical Test

Total number of specimens: 41

Serotype discriminated by invention kit	Number of detected specimen(s)
HU/NLV/Kashiwa 645/1999/JP	1
Hu/NLV/Seto 124/1989/JP	7
Hu/NLV/Funabashi 258/1996/JP	4
Hu/NLV/Chiba 407/1987/JP	1
HU/NLV/Narita 104/1997/JP	4
Hu/NLV/Sanbu 809/1998/JP	12
Hu/NLV/Ichikawa 754/1998/JP	2
Hu/NLV/Chitta 1876/1996/JP	3
Hu/NLV/Kashiwa 47/1997/JP	1
Hu/NLV/Mie 7k/1994/JP	1
Hu/NLV/Osaka 10-25/1999/JP	2
Total number of detected specimens	38 (93%)

As a result, it has been found that according to the SRSV detection method of the present invention, SRSVs can be detected with a probability as high as 93% and their serotypes can also be discriminated.

Further, the serotypes discriminated by the kit of the present invention were consistent with those ascertained by PCR and an analysis of their base sequences (Table 4).

Table 4 Ascertainment of Serotypes

Total number of specimens: 38

Serotype discriminated by invention kit		Number of specimen(s) discriminated in serotype by PCR and analysis of base sequences
HU/NLV/Kashiwa 645/1999/JP	1 Specimens	1
Hu/NLV/Seto 124/1989/JP	7 Specimens	7
Hu/NLV/Funabashi 258/1996/JP	4 Specimens	4
Hu/NLV/Chiba 407/1987/JP	1 Specimen	1
HU/NLV/Narita 104/1997/JP	4 Specimens	4
Hu/NLV/Sanbu 809/1998/JP	12 Specimens	12
Hu/NLV/Ichikawa 754/1998/JP	2 Specimens	2
Hu/NLV/Chitta 1876/1996/JP	3 Specimens	3
Hu/NLV/Kashiwa 47/1997/JP	1 Specimen	1
Hu/NLV/Mie 7k/1994/JP	1 Specimen	1
Hu/NLV/Osaka 10-25/1999/JP	2 Specimens	2

The anti-SRSV antibodies against the SRSVs (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a

carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL. All the dilutions so obtained were mixed. As an alternative, the anti-SRSV antibodies may be diluted after mixing them together. Using the thus-diluted mixture of the anti-SRSV antibodies, a solid-phase anti-SRSV antibody microplate was produced likewise. With respect to 22 fecal specimens from patients infected to SRSV, ELISA was conducted in a similar manner as in Test 1. It was possible to detect SRSV in 20 specimens.

Industrial Applicability

According to the SRSV detection kit of the present invention, it is possible to detect most of the SRSV-related viruses discovered to date and also to discriminate their serotypes and genogroups. When SRSV-related food poisoning occurs, the SRSV detection kit of the present invention is, therefore, useful for specifying an infection route, preventing the infection from spreading, and performing an epidemiological investigation.